Available online at www.sciencedirect.com

SCIENCE DIRECT

Virology 336 (2005) 229 – 239

VIROLOGY

www.elsevier.com/locate/yviro

An arthritogenic alphavirus uses the α1β1 integrin collagen receptor

May La Linn[a,b,c], Johannes A. Eble[d], Christoph Lübken[d], Robert W. Slade[e], Jyrki Heino[f], Janet Davies[g,i], Andreas Suhrbier[a,b,c,*]

[a]*The Australian Centre for International and Tropical Health and Nutrition, Brisbane, Queensland, Australia*
[b]*Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Brisbane, Queensland 4029, Australia*
[c]*University of Queensland, Brisbane, Queensland, Australia*
[d]*Institut für Physiologische Chemie und Pathobiochemie, Universitätsklinikum Münster, Münster, Germany*
[e]*Graduate Research College, Southern Cross University, Lismore, NSW, Australia*
[f]*University of Jyvaskyla, Jyvaskyla, Finland*
[g]*The Alfred Hospital, Melbourne, Vic., Australia*
[i]*Monash University, Melbourne, Vic., Australia*

Received 24 November 2004; returned to author for revision 3 January 2005; accepted 15 March 2005
Available online 19 April 2005

Abstract

Ross River (RR) virus is an alphavirus endemic to Australia and New Guinea and is the aetiological agent of epidemic polyarthritis or RR virus disease. Here we provide evidence that RR virus uses the collagen-binding α1β1 integrin as a cellular receptor. Infection could be inhibited by collagen IV and antibodies specific for the β1 and α1 integrin proteins, and fibroblasts from α1-integrin$^{-/-}$ mice were less efficiently infected than wild-type fibroblasts. Soluble α1β1 integrin bound immobilized RR virus, and peptides representing the α1β1 integrin binding-site on collagen IV inhibited virus binding to cells. We speculate that two highly conserved regions within the cell-receptor binding domain of E2 mimic collagen and provide access to cellular collagen-binding receptors.

Keywords: Ross River virus; Receptor; α1β1 integrin; Collagen IV

Introduction

The genus *Alphavirus* in the family *Togaviridae* represents a group of enveloped, positive-sense, single-stranded RNA, arboviruses comprising over 40 known members, which are maintained in nature by continuous cycles of transmission between hematophagous arthropods and vertebrate hosts (Peters and Dalrymple, 1990). Alphaviruses are found on every continent of the world, and based on phylogenetic analysis, have been grouped into the Sindbis (SN) and recombinant virus group, the Venezuelan and eastern equine encephalitis virus group, the Semliki Forest (SF) virus group, and the fish alphaviruses (Powers et al., 2001). The first two groups largely utilize birds as their natural enzootic vertebrate hosts and segregate from the SF virus group, which usually utilizes mammals (Linn et al., 2001). Several alphaviruses also cause disease in humans primarily as a result of epizootic infections. These include the American encephalitic alphaviruses (Venezuelan, eastern, and western equine encephalitis viruses) (Peters and Dalrymple, 1990) and several viruses in the SF virus group that cause arthritis/arthralgia, principally the Afro-Asian chikungunya virus, the African o'nyong-nyong virus, and the Australasian Barmah Forest (BF) and Ross River (RR) viruses (Suhrbier and Linn, 2004). RR virus is the aetiological agent of epidemic polyarthritis or RR virus disease (RRVD), which afflicts up to 8000 Australians annually. In 1979/1980, an epidemic also swept through several islands of the South Pacific resulting in tens of thousands

* Corresponding author. Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Brisbane, Queensland 4029, Australia. Fax: +61 7 3362 0107.
E-mail address: andreasS@qimr.edu.au (A. Suhrbier).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.03.015

of cases (Harley et al., 2001). The disease is characterized by severe polyarthritis, which resolves over 3–6 months, with about half the patients also experiencing a rash, fever, myalgia, or fatigue (Mylonas et al., 2002). The arthritic symptoms of RRVD are believed to be caused by persistent productive infections of synovial macrophages (Linn et al., 1996, 1998; Mateo et al., 2000; Soden et al., 2000; Suhrbier and Linn, 2004).

The alphavirus genome encodes four non-structural proteins, a capsid protein, and the spike proteins E1 and E2, which are arranged as 240 heterodimers assembled into 80 trimeric spikes on the virus surface. The E1 protein directs the membrane fusion process, and E2 is thought to interact with the cellular receptor for the virus (Hammar et al., 2003). The sequence around amino acids 170–220 of E2 is postulated to constitute a cell-receptor binding domain for SN virus (Myles et al., 2003), whereas the cell-receptor binding domain for RR virus E2 appears to be extended towards the C-terminus and is thought to lie between amino acids 170 and 262 (Davies et al., 2000; Smith et al., 1995; Vrati et al., 1988). DC-SIGN and L-SIGN have been shown to be attachment receptors for SN virus probably by binding virion carbohydrate moieties (Klimstra et al., 2003). Heparin sulphate has been implicated as a binding or capture receptor for a number of alphaviruses including SN, SF, and RR virus, with alphavirus binding to heparin sulphate largely dependent on positively charged amino acids in E2 (Zhang et al., 2005). Increasing heparin sulphate binding affinity often arises from mutations, which arise during propagation of viruses in mammalian tissue culture (Mandl et al., 2001; Smit et al., 2002). However, binding to heparin sulphate is probably insufficient for viral entry into cells, with the entry receptor likely to be a distinct molecular entity (Heil et al., 2001). Such a cellular receptor for SN virus in mammalian cells has been reported as the 67-kDa high-affinity laminin receptor (Wang et al., 1992), and a 32 kDa receptor, which also appeared to be a laminin receptor, was identified as a receptor for Venezuelan equine encephalitis virus in mosquito cells (Ludwig et al., 1996). Unknown 74-kDa and 110-kDa proteins have also been reported as possible receptors for SN virus on mouse neuroblastoma cells (Ubol and Griffin, 1991), as has a 63-kDa protein on chicken cells (Wang et al., 1991). To our knowledge, no cellular receptor for any alphaviruses in the SF group has so far been reported.

Here we provide evidence that the α1β1 integrin (also known as VLA-1 or CD49a/CD29) is a receptor for RR virus on mammalian cells. The α1β1 integrin has a high affinity for type IV collagen and is used by adherent cells to bind to the extracellular matrix. The RR virus E2 sequence between amino acids 170 and 262 contains two conserved regions that may fold to mimic collagen and thereby mediate binding to cellular collagen receptors.

Results

RR virus infection is inhibited by collagen IV and heparin

We have previously noted that RR virus infects most adherent cell lines, but few non-adherent cell lines, suggesting that the cellular RR virus receptor might be involved in binding to the extracellular matrix (ECM) (Linn and Suhrbier, 1997; Linn et al., 1996). To test whether soluble ECM proteins might block RR virus infection and subsequent cytopathic effects (CPE), collagen I, II, and IV, laminin, and fibronectin were incubated with HeLa cells. RR virus (T48) was then added and CPE was measured at 48 h post-infection. The multiplicity of infection (MOI) used in these experiments gave >80% CPE after this period in the absence of ECM proteins. Collagen IV was able to inhibit RR virus-mediated CPE in a dose-dependent manner, whereas laminin, fibronectin, and collagen I and II had no detectable blocking activity (Fig. 1A), suggesting that RR virus utilizes a cellular collagen IV receptor to infect these cells. Collagen IV inhibited both RR virus derived from mammalian HeLa cell cultures (Fig. 1A, RR xHeLa) and RR virus derived from C6/36 mosquito cell cultures (Fig. 1A, RR xC6/36). Infection by BF virus, another arthritogenic virus from the SF virus group (Flexman et al., 1998), was also inhibited by collagen IV, but not laminin or fibronectin (Fig. 1A). Neither infection by SF virus, nor the recently identified Southern elephant seal (SES) virus could be appreciably blocked by laminin, collagen I, or collagen IV (Fig. 1A), illustrating that collagen IV is not a non-specific inhibitor of alphavirus-induced CPE.

Several alphaviruses including RR virus are known to bind heparin by virtue of positively charged amino acids in E2 (Zhang et al., 2005), which are thought to be selected for during extended passage in mammalian cells (Mandl et al., 2001; Smit et al., 2002). To test for heparin binding, virus and heparin were first co-incubated for 1 h before addition to HeLa cell cultures. CPE mediated by HeLa cell-derived RR virus (T48) and BF virus, but not SF or SES virus, could be inhibited by heparin (Fig. 1B). CPE mediated by SN and RR14389, a human RR virus isolate, was also inhibited by heparin (data not shown). After a single passage of RR virus (T48) in C6/36 mosquito cells, a virus population emerged that was no longer inhibited by heparin (Fig. 1B, RR xC6/36). This is consistent with previous studies (Mandl et al., 2001; Smit et al., 2002), where only RR virus derived from passage in mammalian cells could be effectively blocked by heparin. Passage of RR virus in mosquito cells clearly results in rapid loss of heparin binding. Importantly, both infection by RR virus derived from mammalian cells and RR virus derived from C6/36 cells was inhibited by collagen IV (Fig. 1A).

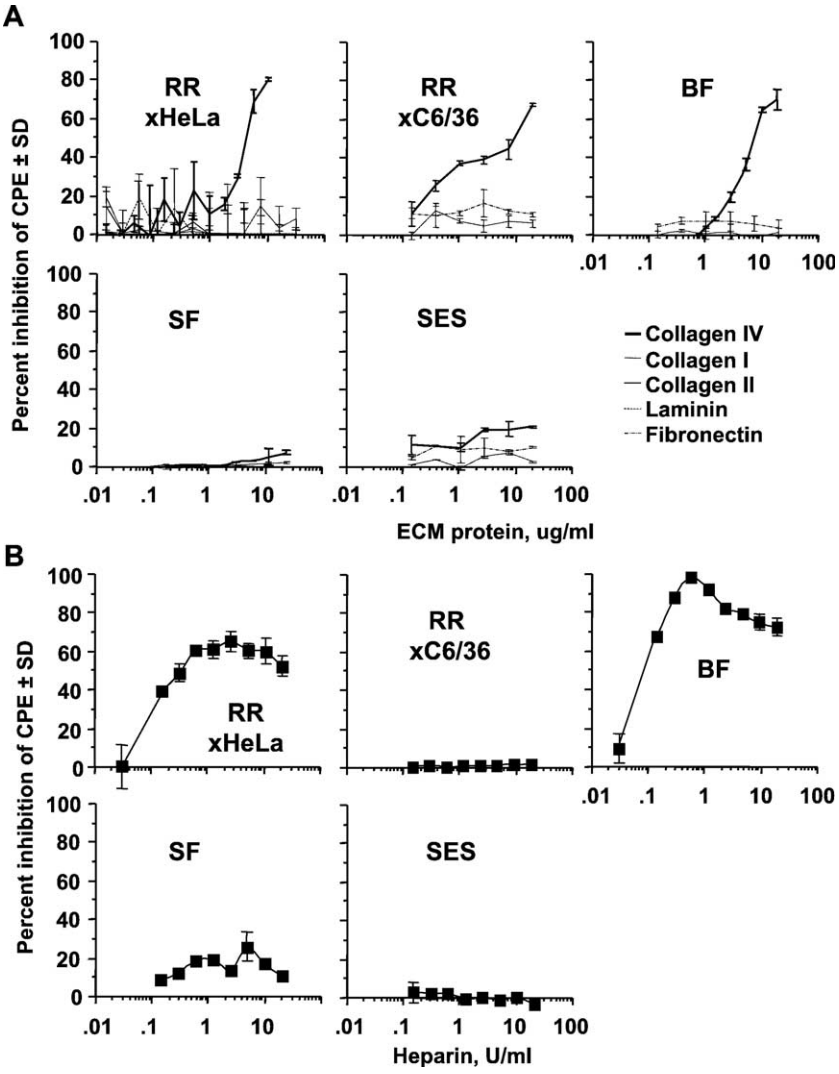

Fig. 1. Inhibition of RR virus-induced cytopathic effect (CPE) by extracellular matrix (ECM) proteins and heparin. HeLa cells were seeded in quadruplicate into 96-well plates and cultured overnight. (A) Collagen, laminin, or fibronectin were then added to the wells for an hour at the indicated concentrations before addition of RR virus derived from HeLa cell cultures (RR xHeLa), RR virus derived from C6/36 cell cultures (RR xC6/36), and BF, SF, or SES virus (derived from HeLa cell cultures). After culture for 48 h at 37 °C and 5% CO_2, the cells remaining attached to the plate were fixed and stained using crystal violet protein stain. The stain was eluted in methanol and read at OD595. An OD reading equivalent to the OD reading from wells without virus meant that no cells were killed by the virus and represents 0% CPE or 100% inhibition of CPE. Zero percent inhibition of CPE means that the blocking agent failed to reduce the CPE or increase the OD compared to the control wells, which contained virus but no blocking agent. These latter wells always showed >80% CPE. (B) Heparin was incubated with the virus for 1 h before addition to the wells containing the cells. This protocol was adopted, since the virus may interact with heparin, whereas collagen interacts with the cells. The plates were then cultured for 48 h and inhibition of CPE calculated as above.

Anti-β1 and anti-α1 antibodies inhibited RR virus-mediated CPE

Cells can bind collagen via a number of heterodimeric integrin receptors. Four integrins are currently recognized as collagen receptors; α1β1 and α2β1, and the recently identified α10β1 and α11β1 integrins (Gullberg and Lundgren-Akerlund, 2002). To determine whether RR virus uses collagen-binding integrins as receptors, a panel of antibodies specific for the β1, β2, α1, and α2 integrin proteins was tested for their ability to inhibit RR virus-mediated CPE of HeLa cells. A monoclonal anti-β1 antibody was able to inhibit RR virus-mediated CPE, but not CPE mediated by SES or SN virus (Fig. 2A, anti-β1 61.2.C4). A monoclonal antibody specific for β2 integrin failed effectively to block RR virus-mediated CPE (Fig. 2A, RR anti-β2 HB203). Monoclonal antibodies specific for α1 (FB12) and α2 (P1E6) were also individually able to inhibit RR virus-induced CPE by about 35%, but had no inhibitory activity against SES virus infections (Fig. 2A, anti-α1 FB12 and anti-α2 P1E6). An anti-α5 (FB12) monoclonal antibody showed no blocking activity against RR or SES virus (Fig. 2A, anti-α5 FB12). An irrelevant anti-CD3 monoclonal antibody also failed to inhibit RR virus-mediated CPE (data not shown). In addition, a polyclonal goat anti-β1 antibody (Fig. 2B, anti-β1 172, black squares) was able to inhibit RR

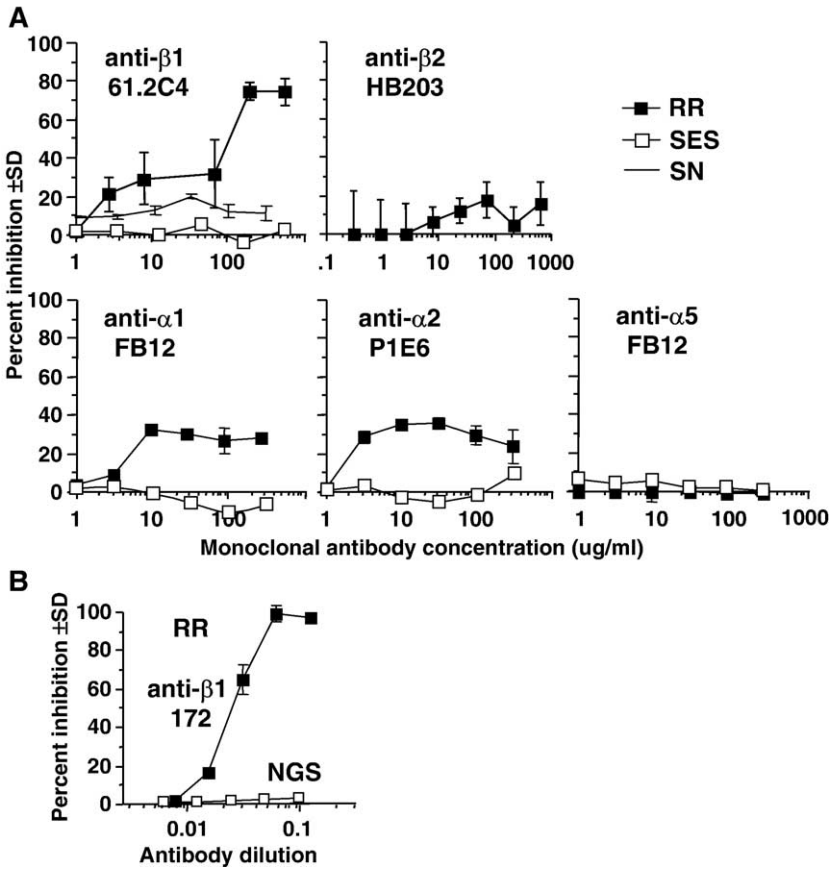

Fig. 2. RR virus-induced CPE is blocked by anti-β1 and anti-α1 antibodies. HeLa cells were set up as for Fig. 1 and antibody incubated with the cells for an hour at the indicated concentrations before addition of virus. Percent inhibition was calculated as for Fig. 1. (A) Testing of blocking monoclonal antibodies specific for integrin subunits (α1, α2, α5, β1, and β2) at the concentrations indicated against RR (black squares), SES (white squares), and SN (line) virus-mediated CPE. (B) Testing of polyclonal goat anti-β1 serum (anti-β1 172) (black squares) and normal goat serum (NGS, white squares) at the indicated serum dilutions against RR virus-mediated CPE.

virus-induced CPE, whereas normal goat serum had no effect (Fig. 2, NGS, white squares).

These experiments indicate that RR virus uses β1 integrin to infect HeLa cells and suggests that both α1β1 and α2β1 integrins may represent receptors used by RR virus to infect these cells. Due to the lack of available reagents, we were unable to explore the role of α10β1 and α11β1 integrins as RR virus receptors.

RR virus binds to soluble α1β1 integrin

To determine whether β1 integrins could also act as binding receptors for RR virus, the ability of RR virus to bind a panel of soluble integrins was investigated using C6/36-derived RR virus (T48). Recombinant purified soluble integrins, devoid of their membrane anchor and cytoplasmic domains, were used to probe RR virus immobilized on ELISA plates. Integrins undergo a conformational change, which increases their affinity for the ligand and the 9EG7 antibody holds the integrin in the high affinity conformation. Furthermore, divalent cations like manganese (Mn2+) are a requirement for integrin binding. Using an anti-β1 integrin antiserum to detect binding of soluble integrins to immobilized RR virus, RR virus was shown to bind soluble α1β1 integrin in the presence of Mn2+ and the 9EG7 antibody (Fig. 3, α1β1 + Mn/9EG7). This binding to RR virus was lost when divalent cations (i.e., Mn2+) were chelated with EDTA, illustrating that the interaction was cation dependent, a characteristic of integrin binding (Fig. 3, α1β1 + EDTA). Strong binding of soluble α1β1 integrin was observed for the collagen IV fragment, CB3, which contains the α1β1 integrin binding site (Eble et al., 1993) (Fig. 3, α1β1 + Mn/9EG7). As expected, this binding was again lost on removal of divalent cations (Fig. 3, α1β1 +EDTA). None of the other soluble integrins tested bound immobilized RR virus, although all bound their known ligands (laminin 1 and 5, and collagen I) in the presence of Mn2+ and 9EG7. None of the soluble integrins bound the negative control ligand, immobilized bovine serum albumin (BSA) (Fig. 3, BSA). These data provided evidence that RR virus can bind α1β1 integrin and might therefore use this receptor as a binding or capture receptor.

The relatively weak binding of RR virus compared to collagen IV in these experiments (Fig. 3) likely reflects the inability of these experiments to mimic the physiological interaction of RR virus with integrin receptors. Much higher

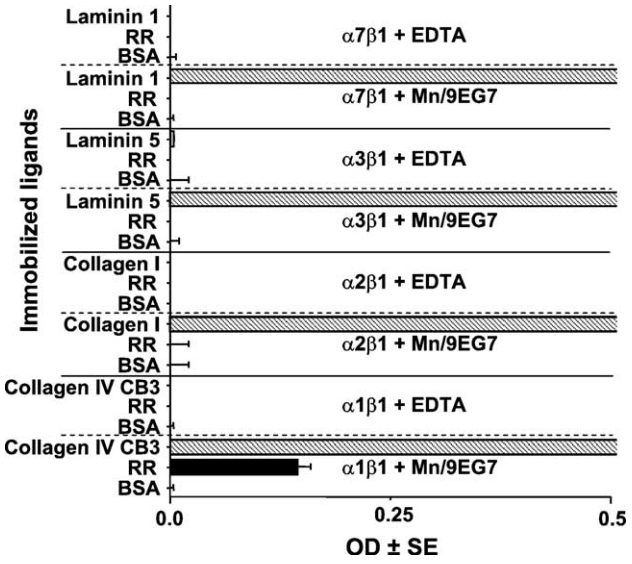

Fig. 3. Soluble α1β1 integrin binds to immobilized RR virus. Laminin, RR virus, bovine serum albumin (BSA), collagen I, or the α1β1-binding fragment of collagen IV, CB3, was immobilized onto ELIZA plates. RR virus binding to the plate was confirmed using a RR virus-specific antibody (see Materials and methods). After blocking, the indicated soluble integrin receptors (15 μg/ml) were added in duplicate for 1 h with EDTA (which prevents the cation dependent integrin binding) or Mn^{2+} (2 mM) plus the monoclonal antibody 9EG7 (which increase the affinity of the integrin for its ligand). After washing, bound integrins were fixed to the wells with 2.5% glutaraldehyde and were detected with a rabbit anti-β1 antiserum, an alkaline phosphatase-labeled secondary antibody, and *p*-nitro-phenylphosphate substrate. Absorbance was measured in an ELISA reader at 405 nm and 595 nm.

avidity interactions are likely to be generated under physiological conditions between multiple E2 binding sites on the virus and the integrin molecules restricted to movement in two dimensions on the cell surface. However, attempts to mimic physiological conditions more effectively by testing for RR virus binding to immobilized integrins in ELISA-based experiments were complicated by the inability to wash away effectively unbound RR virus using washing buffers that necessarily needed to be free of EDTA and detergents (data not shown).

FACS-based assay for blocking of RR virus binding

To further explore whether α1β1 integrin might be involved as a RR virus binding receptor, a FACS-based assay was developed whereby RR virus (T48) bound to cells was detected using a non-neutralizing anti-RR virus monoclonal antibody, D7. The α1β1 integrin binding site has recently been mapped to a small region within the CB3 fragment of collagen IV and was shown to be centered around critical aspartic acid (D) residues in the α1 chains and an arginine (R) residue on the α2 chain of collagen IV (Golbik et al., 2000). Short synthetic peptides representing this binding site on collagen IV, PGPPGDQGPP from the α1 chains and GAKGRAGFP from α2 (critical residues underlined), were able to inhibit binding of RR virus to HeLa cells (Fig. 4). A panel of other peptides representing RR virus E2 sequences failed to inhibit binding (TPPDIPDRTL—Fig. 4; IPDRTLLSQT, RTLLSQTAGN, and YQLTTAPTDE—data not shown). Thus collagen IV peptides representing the α1β1 integrin binding site were able to inhibit RR virus binding to cells, further implicating the α1β1 integrin as a RR virus cell-binding receptor.

RR virus (T48) grown in HeLa cells was used for these experiments. However, since the HeLa cells used in the FACS experiment were treated with trypsin prior to incubation with RR virus, most of the cell-associated heparin is likely to have been removed (Kobayashi et al., 2000). Hence heparin is not excluded as a capture receptor by these experiments.

Although PGPPGDQGPP and GAKGRAGFP could inhibit binding in these FACS assays, we have been unable to demonstrate significant inhibition of RR virus-mediated CPE using these peptides in an experiment similar to those shown in Fig. 1A (data not shown). We believe that our collagen peptides may have insufficient affinity for α1β1

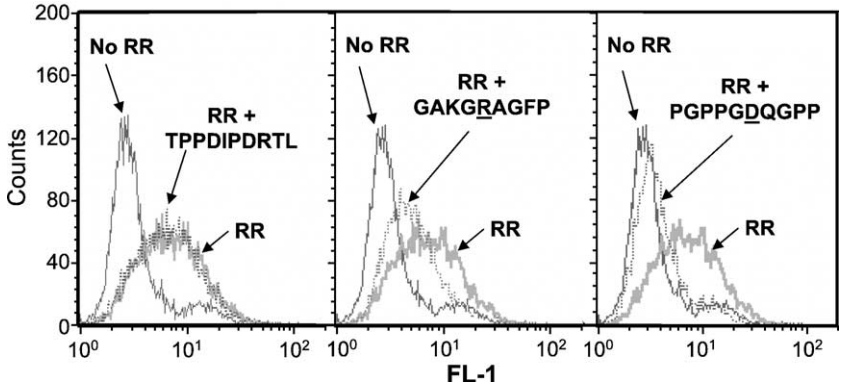

Fig. 4. Collagen IV peptides representing the α1β1-binding site block RR virus binding. FACS profiles of RR virus bound to HeLa cells detected using the non-neutralizing anti-RR virus monoclonal antibody (D7). HeLa cells were (i) incubated without peptide and without RR virus followed by staining with D7 and secondary antibody (No RR), (ii) incubated without peptide and with RR virus followed by staining with D7 and secondary antibody (RR), and (iii) incubated with peptide followed by RR virus and staining with D7 and secondary antibody (RR + peptide). Peptide sequences are indicated in single letter amino acid code. Underlined amino acids illustrate the crucial residues involved in α1β1-binding.

integrin as they do not adequately mimic the heterotrimeric collagen helix (Renner et al., 2004), which represents the α1β1 integrin binding site (Eble et al., 1993).

Expression of α1 integrin correlates with RR virus infection

To further investigate the importance of α1β1 integrin in RR virus infection, embryonic fibroblasts from α1 integrin deficient (α1-KO) mice (Gardner et al., 1999) were infected with serial 10-fold dilutions of different alphaviruses and the cells fixed 3–5 days later before full CPE were observed in wells receiving the least diluted virus ($\log_{10}^{-1}$ virus dilution). RR virus T48 derived from HeLa cells, RR virus T48 derived from C6/36 cells, and RR14389 virus (derived from HeLa) showed significantly lower CPE at $\log10^{-4}$, $\log10^{-4}$, and $\log10^{-5}$ virus dilutions, respectively, on fibroblasts from α1-KO mice compared with control fibroblasts from wild-type mice (Fig. 5, RR T48 xHeLa, RR T48 xC6/36, and RR14389). BF virus showed slightly less CPE in α1-KO fibroblasts, whereas SN and SES and SN virus showed similar or marginally more CPE in α1-KO fibroblasts (Fig. 5). These studies not only support the view that RR virus uses α1β1 integrin as a receptor, but also illustrated that RR virus is able use other receptors in these embryonic cells. As the virus concentrations increase, the lack of α1 integrin has less effect on CPE, suggesting that other receptors compensate for loss of α1 integrin when the MOI increases. To determine whether anti-murine integrin antibodies could further reduce infection of α1-KO embryo fibroblasts, anti-murine α1 (Ha31/8), anti-murine α2 (HMa2), anti-murine β1 (Ha2/5), and anti-murine β2 (GAME-46) were tested in experiments similar to those described in Fig. 2. None of these antibodies showed any ability to further reduce infectivity of α1 KO fibroblasts by RR virus or to reduce infectivity of these cells by the other alphaviruses (data not shown). These experiments suggest that these embryonic murine cells have receptors for alphaviruses that do not involve β1 or β2 integrins. It might be noted that RR virus infection of new born mice is associated with paralysis and muscle pathology, whereas infection of adult mice is asymptomatic (Lidbury et al., 2000; Vrati et al., 1986).

A number of non-adherent human cell lines (Mono Mac 6, HL-60, K562), which are poorly infected by RR virus, show no or weak staining for α1 and α2 integrin by FACS analysis (data not shown).

Discussion

This paper provides evidence that RR virus utilizes the collagen IV-binding β1 integrin, α1β1, as a receptor in mammalian cells. Integrins are well recognized as receptors for a number of unrelated viruses including rotavirus, hantavirus, echovirus, adenovirus, coxsackie virus, cytomegalovirus, foot and mouth disease virus, and West Nile virus (Chu and Ng, 2004; Duque et al., 2004; Feire et al., 2004; Gavrilovskaya et al., 1999; White, 1993). However, to our knowledge, this represents the first report of a virus utilizing α1β1 integrin as a receptor. Collagen-binding β1 integrins are nearly universally expressed on adherent cells (Gullberg and Lundgren-Akerlund, 2002), and are also well conserved in vertebrates (Hynes, 1992). The ability of RR virus to infect most adherent mammalian cells (Linn et al., 1996) and many different vertebrate species is consistent with the use of this receptor. However, like rotaviruses (Guerrero et al., 2000; Londrigan et al., 2000), RR virus appears able to use more than one receptor. Additional receptors may include the collagen-binding integrin receptors (α1β1, α2β1, α10β1, and α11β1), integrins, or non-integrin

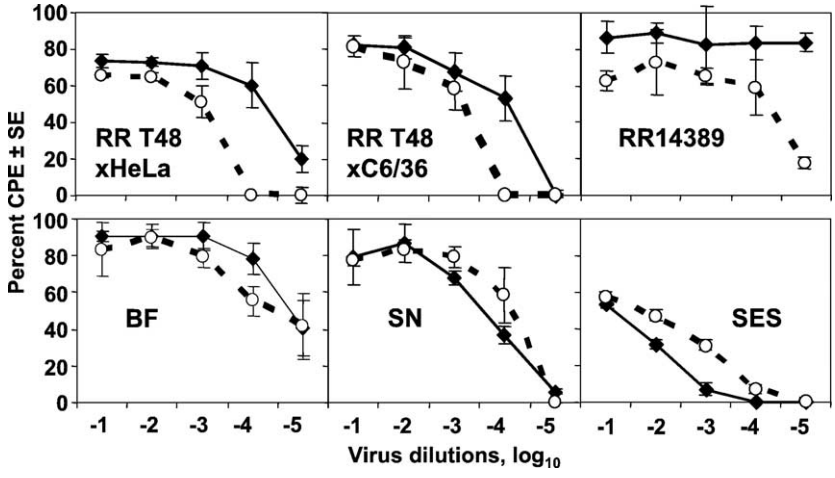

Fig. 5. Reduced RR virus-mediated cytopathic effect (CPE) on embryonic fibroblasts derived from $\alpha1^{-/-}$ mice. Fibroblasts from wild-type control (black diamonds) and $\alpha1^{-/-}$ (white circle) mice were infected with 10-fold serial dilutions of six alphaviruses and after 5 days the level of CPE was measured using crystal violet staining and expressed as a percentage of control uninfected cells. For RRV T48 derived from HeLa cells at $\log10^{-4}$ virus dilution, the percentage CPE for $\alpha1^{-/-}$ and wild-type fibroblasts was significantly different ($P = 0.001$, unpaired Student's t test). This was also the case for RR virus T48 derived from C6/36 cells at $\log10^{-4}$ virus dilution ($P = 0.049$) and RR14389 at $\log10^{-5}$ virus dilution ($P = 0.037$).

collagen receptors (Gullberg and Lundgren-Akerlund, 2002), with infection of murine embryonic fibroblasts able to occur independently of β1 or β2 integrins.

The cell-receptor binding domain for RR virus E2 appears to lie between amino acids 170 and 262, and phage display analysis has placed components of the recognition sites of two RR virus neutralizing antibodies around E2 172–182 (PPDIPDRTLLS) and E2 234–248 (KWQFTSPFVPRADQT) (Davies et al., 2000). Interestingly, these two regions include a number of highly conserved amino acids in the SF group of alphaviruses that use mammals as their enzootic vertebrate hosts (Fig. 6, underlined sequences). Furthermore, these E2 regions show some similarities with collagen IV sequences known to act as the α1β1 integrin binding site, PGPPGDQGPP (from the α1 chain of collagen) and GAKGRAGFP (from the α2 chain of collagen). The E2 and collagen IV sequences both contain the characteristic P and/or G residues of collagen and the crucial R and D residues (underlined), which in the collagen sequences are known to be required for α1β1 integrin binding to collagen IV (Golbik et al., 2000). This level of homology may not appear high. However, the multiple ligands for α1β1 and α2β1 integrins, which include several collagen types, laminin, certain virus receptors (Graham et al., 2003), and certain snake venoms (Eble and Tuckwell, 2003), also only show limited sequence homology.

The ability to block infection using the CPE assays described herein probably requires both high-affinity blocking agents and restricted use by the virus of other receptors that can compensate for loss of the receptor targeted by the blocking agent. The inability of collagen I to block RR virus infection probably reflects its low affinity for the α1β1 integrin (Tulla et al., 2001). In contrast, collagen IV, which has a high affinity for α1β1 integrin (Tulla et al., 2001), was clearly able to mediate detectable blocking (Fig. 1A). Even collagen I's affinity for its preferred receptor, the α2β1 integrin, may be too low to mediate effective blocking, since anti-α2 antibody, but not collagen I, partially inhibited RR virus infection (Figs. 1A and 2). To our knowledge, there are no reports of collagen I inhibiting infection of viruses known to use α2β1 integrin as a receptor, further supporting the view that standard collagen I preparations may not be useful reagents for blocking virus infection via the α2β1 integrin receptor. The relatively low affinity interaction between collagen IV and the α2β1 integrin probably also renders collagen IV unable to block effectively infection via the α2β1 integrin in such experiments. The inability of collagen IV to block effectively infection of SF and SES virus (Fig. 1B) does not provide evidence that these viruses do not utilize the α1β1 integrin, since for these viruses other receptors (e.g., α2β1 integrin) may be able to compensate for loss of infection via the α1β1 integrin. RR and BF viruses appear to have a preference for α1β1 integrin, with no other receptors present on HeLa cells able to compensate sufficiently when this receptor is blocked.

It is tempting to speculate that the use of collagen receptors by RR virus and BF virus is related to the induction of polyarthritic disease by these viruses in humans. Not all individuals infected with RR virus develop RRVD (Harley et al., 2001) and the factors that predispose to symptomatic seroconversion remain elusive. Expression of platelet α2β1 integrin (GPIa/IIa) has been shown to vary up to 10-fold in different individuals due to allelic variation in the gene encoding α2 (Kritzik et al., 1998), and β3 integrin (GPIIIa) contains polymorphisms that effect its function (Kunicki and Nugent, 2002). Although to date no such polymorphisms have been identified in α1β1 integrin genes, genetic variation in RR virus receptors might influence predisposition to symptomatic RR virus infection. However, perhaps a more compelling association between α1β1 integrin usage and arthritis comes from recent studies showing that anti-α1 and α2-integrin antibodies can block several in vivo models of inflammation including anti-

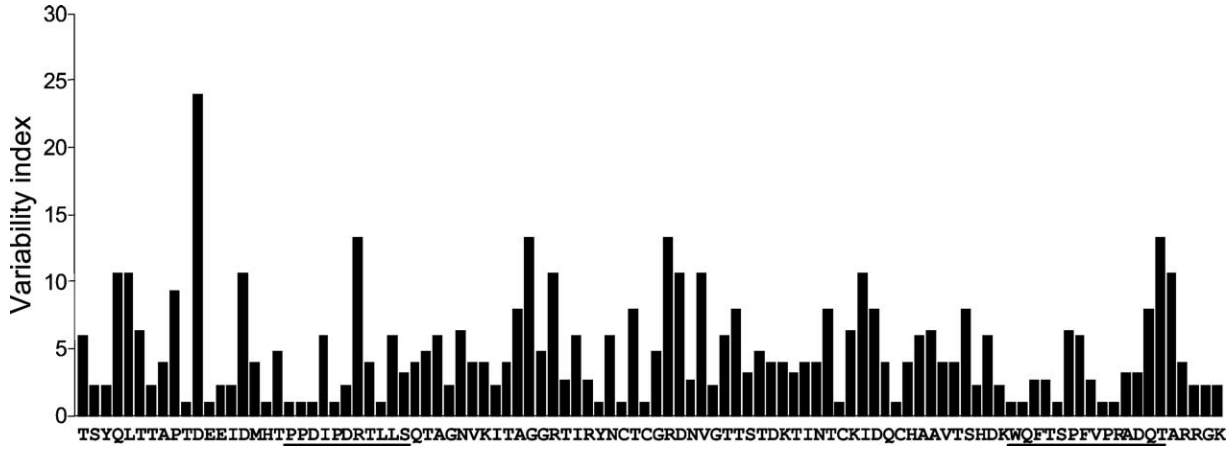

Fig. 6. Wu Kabat analysis of region of E2 from the SF virus group believed to be involved in receptor binding. The E2 sequence of RR/1 (154–253) is shown and the variability index calculated, as described (Wu and Kabat, 1970), from the following sequences; BF (AAB40702), chikungunya virus (L37661), Igbo Ora virus (AAC97207), o'nyong-nyong (AAC97205), RR/1 (AAA47404), RR/2 (P08491), Sagiyama virus (BAA92847), SF (X78112), and SES (AF315122). The underlined sequences are postulated to be involved in contact with the cellular receptor (Davies et al., 2000).

collagen antibody-induced arthritis, possibly via blocking of migration or activation of monocytes (de Fougerolles et al., 2000). The arthritic symptoms of RRVD are associated with inflammatory synovial infiltrates comprised primarily of monocytes and macrophages (Fraser et al., 1981). RR virus has also been detected in such infiltrates in vivo many weeks after disease onset (Soden et al., 2000) and RR virus can establish persistent productive cytopathic infections in macrophage cultures in vitro (Linn and Suhrbier, 1997; Suhrbier and Linn, 2004; Way et al., 2002). Furthermore, such cultures secrete IL-8 (CXCL8), monocyte chemoattractant protein-1 (CCL2), and macrophage inflammatory protein-2 (CXCL2) (Mateo et al., 2000; Way et al., 2002), chemokines involved in monocyte recruitment and activation (Gerszten et al., 1999). Monocytes express only low levels of α1β1, but upon activation and differentiation into macrophages expression is rapidly upregulated (Rubio et al., 1995), consistent with the ability of RR virus to infect macrophages, but not monocytes in vitro (Linn et al., 1996). Development of arthritis may be dependent on access of RR virus into joint tissues, a process that might be facilitated by the existence during the short viremic period of subclinical inflammatory joint lesions containing macrophages with high α1β1 integrin expression. Infection and/or viral persistence in the joint may also be expedited by the continued recruitment into the inflamed joint of monocytes that rapidly upregulate expression of a RR virus receptor.

Materials and methods

Cells

HeLa cells (ATCC CCL-2) were propagated in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 10 mM HEPES, 100 U/ml penicillin G sodium, and 100 μg/ml streptomycin sulphate. K-562 (ATCC CCL-243), Mono Mac 6 (Ziegler-Heitbrock et al., 1988), and HL-60 (ECCC 88120805) were cultured as above in medium shown to contain <0.05 ng/ml lipopolysaccharide (Sweet and Hume, 1995), equivalent to <0.025 endotoxin units by Limulus Amebocyte Lysate assay (BioWhitiker). Murine fibroblast cell lines derived from integrin alpha1$^{-/-}$ mice (Gardner et al., 1999) and control wild-type mice were cultured in Dulbecco's modified Eagle's medium supplemented with 15% fetal bovine serum (FBS), 2 mM L-glutamine, 10 mM HEPES, 0.01 mM MEM non-essential amino acids, and antibiotics. The vertebrate cell lines were cultured at 37 °C in 5% CO_2. The invertebrate C6/36 cells (ATCC CRL 1660) were grown in RPMI 1640 media supplemented with 10% FBS, 0.01 mM MEM non-essential amino acids, 2 mM L-glutamine, 10 mM HEPES, and antibiotics, and were incubated at 30 °C in 5% CO_2. All cell culture reagents were purchased from Gibco, Invitrogen, Melbourne, Victoria, Australia.

Alphaviruses

Stocks of (i) RR virus T48 strain, (ii) RR virus 14389 strain (Doherty et al., 1972), (iii) Semliki Forest virus (supplied by P. Hertzog, Monash University, Victoria, Australia), (iv) SN virus (provided by R. Hall, University of Queensland, Queensland, Australia), (v) Barmah Forest (provided by B. Kay, Queensland Institute of Medical Research), and (vi) Southern elephant seal virus (SES) (Linn et al., 2001) were prepared by infecting a subconfluent monolayer of HeLa cells or C6/36 cells in T75 culture flasks (Corning). The cell culture fluid was harvested between 18 and 26 h and centrifuged at 1000 × *g* (Beckman GS-15R centrifuge) for 30 min at 4 °C to remove cellular debris. The supernatant was dispensed into small aliquots and stored at −70 °C. Polyethylene glycol (PEG)-purified RR virus (T48) was a generous gift from PanBio Pty Ltd, Australia. The purified RR virus was inactivated by exposure to 960 μW/cm^2 of UV-C for 2 h (Linn et al., 1996) prior to use in the soluble integrin binding ELISA. All cells lines and viruses were negative for mycoplasma (Linn et al., 1995).

Inhibition by of virus-induced cytopathic effect (CPE)

The dilutions of virus stocks that killed >80% of cells in 48 h were used in these assays. Virus was added in quadruplicate into 96-well plates seeded with 10^4 HeLa cells on the previous day. CPE was determined 48 h post-infection by fixation and staining of infected and uninfected control cells with 0.05% crystal violet and 10% formaldehyde in phosphate-buffered saline. After washing with water and air drying, the level of CPE was measured by eluting the crystal violet stain taken up by living cells with 100% methanol and reading at OD595 ($VERSA_{max}$ microplate reader, Molecular Devices). Percent killing was calculated as (mean OD of uninfected cell control wells [without virus]) − (mean OD of wells infected with virus) / (mean OD of uninfected cell control wells [without virus]). Percent inhibition of CPE was calculated as (percentage killing of cells by virus alone) − (percentage killing of cells with blocking agent)/(percentage killing of cells by virus alone).

For inhibition by extracellular matrix proteins, HeLa cells were incubated at room temperature for 1 h with serial dilutions of (i) bovine collagen I or bovine collagen II prepared as described previously (Rowley et al., 1986), (ii) collagen IV from Engelbreth–Holm–Swarm murine sarcoma basement membrane (Sigma), (iii) fibronectin from human plasma (Sigma), or (iv) laminin from human placenta (Sigma). Virus was then added and the cells cultured for 48 h. For inhibition by heparin, virus was incubated with serial dilutions of sodium heparin (from porcine and bovine mucous, Pharmacia and Upjohn Pty Ltd, Perth, Australia) at room temperature for 1 h before addition of 100 μl of the heparin virus mixture to a HeLa cell

monolayer in 100 μl of medium, followed by incubation for 48 h before fixation.

For inhibition by anti-integrin antibodies, HeLa cells were incubated with serial dilutions of anti-integrin antibodies at room temperature for 1 h and followed by addition of virus and culture for 48 h. The following blocking anti-integrin monoclonal antibodies were used: antihuman integrin α1 (FB12), antihuman integrin α2 (P1E6), antihuman integrin α5 (FB12) (Chemicon Australia Pty Ltd), antihuman integrin β1 (61.2C4), and antihuman integrin β2, (HB203) (generous gifts from J. Gamble, the Hanson Institute, Adelaide, South Australia). The goat polyclonal antihuman β1 antibody (serum 172) was provided by M. Ginsberg (Scripps Research Institute, La Jolla, USA). The control monoclonal antibody was anti-CD3 (IOT3b) (Immunotech S.A., Marseille, France). The blocking anti-murine integrin antibodies were anti-murine α1 (Ha31/8), anti-murine α2 (HMa2), anti-murine β1 (Ha2/5), and anti-murine β2 (GAME-46) (BD Biosciences Pharmingen).

Blocking of RR virus binding assay

A single cell suspension of HeLa cells was prepared by treating HeLa cell monolayers with trypsin-EDTA and washing once with phosphate-buffered saline containing 2% FCS (PBS-2). Cell pellets of 2×10^5 cells were incubated with 40 μl of 2 mg/ml of the following peptides, PGPPGDQGPP, GAKGRAGFP (Collagen IV), TPPDIPDRTL, IPDRTLLSQT, RTLLSQTAGN, and YQLTTAPTDE (RR virus E2) (Chiron Technologies, Clayton, Victoria, Australia), for 1 h at 4 °C and followed by incubation with 10 μl of RR virus (virus to cell ratio of 10 $CCID_{50}$:1) for 1 h at 4 °C. Unbound virus was washed off with PBS-2 and HeLa cell-bound RR virus was detected by incubating with 1:10 dilution of an anti-RR virus D7 antibody hybridoma culture supernatant for 30 min at 4 °C; (D7 is a non-neutralizing anti-RR virus mouse monoclonal antibody provided by J. Aaskov, Queensland University of Technology, Queensland, Australia). After three washes with PBS-2, cells were incubated with FITC-conjugated sheep $F(ab')_2$ anti-mouse (Silenus, Australia) for 30 min at 4 °C, washed twice, and analyzed by FACScan flow cytometer (Becton Dickinson) using the cellQuest software.

Soluble integrin binding ELISA assay

Preparation and purification of recombinant soluble integrins, and the soluble integrin binding ELISA assay, were carried out at the Institut für Physiologische Chemie und Pathobiochemie, Germany, as described previously (Eble and Tuckwell, 2003; Eble et al., 1998, 2001; Golbik et al., 2000). Microtiter plate wells (Greiner, Nuertingen, Germany) were coated at 4 °C overnight with 100 μl of 10 μg/ml of CB3 (Vandenberg et al., 1991), bovine collagen-1 (prepared by standard protocol), human laminin-1 (a kind gift from R Timpl, Max-Planck-Institute for Biochemistry, Munich, Germany), human laminin-5 (Rousselle et al., 1991), or UV-inactivated purified RR virus in 50 mM Tris–HCl, pH 7.4, 150 mM NaCl (TBS). After washing three times with TBS containing 2 mM $MgCl_2$ (TBS-Mg), the wells were blocked with 1% denatured bovine serum albumin (Sigma) in TBS-Mg (BSA-TBS-Mg) for 2 h at room temperature. The presence of immobilized RR virus was confirmed by ELISA using a polyclonal rabbit anti-RR virus (T48) antibody (kind gift from Dr Aaskov, Queensland University of Technology, Australia), which had a neutralization index of 5.5 at 1:10 dilution (data not shown). When used at 1:100 dilution followed by anti-rabbit HRP and ABTS substrate (Sigma), RR virus-coated plates generated an OD 405 of 1.7 ± 0.2 SD, whereas control sera gave an OD of 0.25 ± 0.05. After blocking, the soluble integrins (15 μg/ml diluted in TBS-Mg containing 1 mM $MnCl_2$) and activating anti-β1 rat MAb 9EG7 (kindly provided by D. Vestweber, University Munster, Germany) were added to the plates for 2 h at room temperature in the presence and absence of 10 mM EDTA. The wells were washed twice with HEPES buffer (50 mM HEPES, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM $MnCl_2$ with or without 10 mM EDTA). The bound integrins were then fixed to the wells with 2.5% glutaraldehyde in HEPES buffer for 10 min at room temperature and washed three times with TBS-Mg. The bound soluble integrins were then detected using rabbit anti-β1 antiserum (Eble and Tuckwell, 2003) (diluted 1:400 in BSA-TBS-Mg) and alkaline phosphatase-conjugated anti-rabbit IgG (1:600 in BSA-TBS-Mg) (Sigma) for 1.5 h each at room temperature, with three washes with TBS-Mg after each incubation. The color was developed with *p*-nitrophenylphosphate (pNpp) (Sigma) in a 0.1 M glycine-buffer, pH 10.4, supplemented with 1 mM $MgCl_2$ and 1 mM Zn-acetate. The color reaction was stopped by addition of 1.5 M NaOH solution, and absorbance was measured in an ELISA reader (Dynatech, Germany) at 405 nm and 595 nm (Eble and Tuckwell, 2003).

Surface expression of integrin receptors

Cells (2×10^5) were washed with PBS-2 and blocked with 5% normal human immunoglobulin (CSL Pty Ltd, Australia) for 25 min at 4 °C, followed by incubation with 2 μg of anti-integrin (FB12 or P1E6) or isotype control monoclonal antibody for 30 min at 4 °C. After three washes with PBS-2, cells were incubated with anti-mouse IgG-FITC for 30 min at 4 °C and analyzed by FACS as above.

Acknowledgments

The authors would like to thank Dr. J. Gamble, Hanson Institute, South Australia, for help with this project. This work was supported by the National Health and Medical Research Council of Australia, the Australian Centre for

International and Tropical Health and Nutrition, and the German Research Council (DFG-grant Eb177/3-2).

References

Chu, J.J., Ng, M.L., 2004. Interaction of West Nile virus with alpha v beta 3 integrin mediates virus entry into cells. J. Biol. Chem. 279, 54533–54541.

Davies, J.M., Cai, Y.P., Weir, R.C., Rowley, M.J., 2000. Characterization of epitopes for virus-neutralizing monoclonal antibodies to Ross River virus E2 using phage-displayed random peptide libraries. Virology 275, 67–76.

de Fougerolles, A.R., Sprague, A.G., Nickerson-Nutter, C.L., Chi-Rosso, G., Rennert, P.D., Gardner, H., Gotwals, P.J., Lobb, R.R., Koteliansky, V.E., 2000. Regulation of inflammation by collagen-binding integrins alpha1beta1 and alpha2beta1 in models of hypersensitivity and arthritis. J. Clin. Invest. 105, 721–729.

Doherty, R., Carley, J., Best, J., 1972. Isolation of Ross River virus from man. Med. J. Aust. 1, 1083S–1084S.

Duque, H., LaRocco, M., Golde, W.T., Baxt, B., 2004. Interactions of foot-and-mouth disease virus with soluble bovine alphaVbeta3 and alphaV-beta6 integrins. J. Virol. 78, 9773–9781.

Eble, J.A., Tuckwell, D.S., 2003. The alpha2beta1 inhibitor rhodocetin binds to the A-domain of the integrin alpha2 subunit proximal to the collagen binding site. Biochem. J. 376, 77–85.

Eble, J.A., Golbik, R., Mann, K., Kuhn, K., 1993. The alpha 1 beta 1 integrin recognition site of the basement membrane collagen molecule [alpha 1(IV)]2 alpha 2(IV). EMBO J. 12, 4795–4802.

Eble, J.A., Wucherpfennig, K.W., Gauthier, L., Dersch, P., Krukonis, E., Isberg, R.R., Hemler, M.E., 1998. Recombinant soluble human alpha 3 beta 1 integrin: purification, processing, regulation, and specific binding to laminin-5 and invasin in a mutually exclusive manner. Biochemistry 37, 10945–10955.

Eble, J.A., Beermann, B., Hinz, H.J., Schmidt-Hederich, A., 2001. Alpha 2beta 1 integrin is not recognized by rhodocytin but is the specific, high affinity target of rhodocetin, an RGD-independent disintegrin and potent inhibitor of cell adhesion to collagen. J. Biol. Chem. 276, 12274–12284.

Feire, A.L., Koss, H., Compton, T., 2004. Cellular integrins function as entry receptors for human cytomegalovirus via a highly conserved disintegrin-like domain. Proc. Natl. Acad. Sci. U.S.A. 101, 15470–15475.

Flexman, J.P., Smith, D.W., Mackenzie, J.S., Fraser, J.R., Bass, S.P., Hueston, L., Lindsay, M.D., Cunningham, A.L., 1998. A comparison of the diseases caused by Ross River virus and Barmah Forest virus. Med. J. Aust. 169, 159–163.

Fraser, J.R., Cunningham, A.L., Clarris, B.J., Aaskov, J.G., Leach, R., 1981. Cytology of synovial effusions in epidemic polyarthritis. Aust. N.Z.J. Med. 11, 168–173.

Gardner, H., Broberg, A., Pozzi, A., Laato, M., Heino, J., 1999. Absence of integrin alpha1beta1 in the mouse causes loss of feedback regulation of collagen synthesis in normal and wounded dermis. J. Cell Sci. 112, 263–272.

Gavrilovskaya, I.N., Brown, E.J., Ginsberg, M.H., Mackow, E.R., 1999. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. J. Virol. 73, 3951–3959.

Gerszten, R.E., Garcia-Zepeda, E.A., Lim, Y.C., Yoshida, M., Ding, H.A., Gimbrone, M.A. Jr., Luster, A.D., Luscinskas, F.W., Rosenzweig, A., 1999. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 398, 718–723.

Golbik, R., Eble, J.A., Ries, A., Kuhn, K., 2000. The spatial orientation of the essential amino acid residues arginine and aspartate within the alpha1beta1 integrin recognition site of collagen IV has been resolved using fluorescence resonance energy transfer. J. Mol. Biol. 297, 501–509.

Graham, K.L., Halasz, P., Tan, Y., Hewish, M.J., Takada, Y., Mackow, E.R., Robinson, M.K., Coulson, B.S., 2003. Integrin-using rotaviruses bind alpha2beta1 integrin alpha2 I domain via VP4 DGE sequence and recognize alphaXbeta2 and alphaVbeta3 by using VP7 during cell entry. J. Virol. 77, 9969–9978.

Guerrero, C.A., Mendez, E., Zarate, S., Isa, P., Lopez, S., Arias, C.F., 2000. Integrin alpha(v)beta(3) mediates rotavirus cell entry. Proc. Natl. Acad. Sci. U.S.A. 97, 14644–14649.

Gullberg, D.E., Lundgren-Akerlund, E., 2002. Collagen-binding I domain integrins—what do they do? Prog. Histochem. Cytochem. 37, 3–54.

Hammar, L., Markarian, S., Haag, L., Lankinen, H., Salmi, A., Cheng, R.H., 2003. Prefusion rearrangements resulting in fusion Peptide exposure in Semliki forest virus. J. Biol. Chem. 278, 7189–7198.

Harley, D., Sleigh, A., Ritchie, S., 2001. Ross River virus transmission, infection, and disease: a cross-disciplinary review. Clin. Microbiol. Rev. 14, 909–932.

Heil, M.L., Albee, A., Strauss, J.H., Kuhn, R.J., 2001. An amino acid substitution in the coding region of the E2 glycoprotein adapts Ross River virus to utilize heparan sulfate as an attachment moiety. J. Virol. 75, 6303–6309.

Hynes, R.O., 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69, 11–25.

Klimstra, W.B., Nangle, E.M., Smith, M.S., Yurochko, A.D., Ryman, K.D., 2003. DC-SIGN and L-SIGN can act as attachment receptors for alphaviruses and distinguish between mosquito cell- and mammalian cell-derived viruses. J. Virol. 77, 12022–12032.

Kobayashi, H., Hirashima, Y., Sun, G.W., Fujie, M., Nishida, T., Takigawa, M., Terao, T., 2000. Identity of urinary trypsin inhibitor-binding protein to link protein. J. Biol. Chem. 275, 21185–21191.

Kritzik, M., Savage, B., Nugent, D.J., Santoso, S., Ruggeri, Z.M., Kunicki, T.J., 1998. Nucleotide polymorphisms in the alpha2 gene define multiple alleles that are associated with differences in platelet alpha2 beta1 density. Blood 92, 2382–2388.

Kunicki, T.J., Nugent, D.J., 2002. The influence of platelet glycoprotein polymorphisms on receptor function and risk for thrombosis. Vox Sang. 83, 85–90.

Lidbury, B.A., Simeonovic, C., Maxwell, G.E., Marshall, I.D., Hapel, A.J., 2000. Macrophage-induced muscle pathology results in morbidity and mortality for Ross River virus-infected mice. J. Infect. Dis. 181, 27–34.

Linn, M.L., Suhrbier, A., 1997. Persistence of Ross River virus in macrophages. Arbovirus Res. Austr. 7, 153–159.

Linn, M.L., Bellett, A.J., Parsons, P.G., Suhrbier, A., 1995. Complete removal of mycoplasma from viral preparations using solvent extraction. J. Virol. Methods 52, 51–54.

Linn, M.L., Aaskov, J.G., Suhrbier, A., 1996. Antibody-dependent enhancement and persistence in macrophages of an arbovirus associated with arthritis. J. Gen. Virol. 77, 407–411.

Linn, M.L., Mateo, L., Gardner, J., Suhrbier, A., 1998. Alphavirus-specific cytotoxic T lymphocytes recognize a cross-reactive epitope from the capsid protein and can eliminate virus from persistently infected macrophages. J. Virol. 72, 5146–5153.

Linn, M.L., Gardner, J., Warrilow, D., Darnell, G.A., McMahon, C.R., Field, I., Hyatt, A.D., Slade, R.W., Suhrbier, A., 2001. Arbovirus of marine mammals: a new alphavirus isolated from the elephant seal louse, *Lepidophthirus macrorhini*. J. Virol. 75, 4103–4109.

Londrigan, S.L., Hewish, M.J., Thomson, M.J., Sanders, G.M., Mustafa, H., Coulson, B.S., 2000. Growth of rotaviruses in continuous human and monkey cell lines that vary in their expression of integrins. J. Gen. Virol. 81, 2203–2213.

Ludwig, G.V., Kondig, J.P., Smith, J.F., 1996. A putative receptor for Venezuelan equine encephalitis virus from mosquito cells. J. Virol. 70, 5592–5599.

Mandl, C.W., Kroschewski, H., Allison, S.L., Kofler, R., Holzmann, H., Meixner, T., Heinz, F.X., 2001. Adaptation of tick-borne encephalitis virus to BHK-21 cells results in the formation of multiple heparan

sulfate binding sites in the envelope protein and attenuation in vivo. J. Virol. 75, 5627–5637.
Mateo, L., Linn, M.L., McColl, S.R., Cross, S., Gardner, J., Suhrbier, A., 2000. An arthrogenic alphavirus induces monocyte chemoattractant protein-1 and interleukin-8. Intervirology 43, 55–60.
Myles, K.M., Pierro, D.J., Olson, K.E., 2003. Deletions in the putative cell receptor-binding domain of Sindbis virus strain MRE16 E2 glycoprotein reduce midgut infectivity in *Aedes aegypti*. J. Virol. 77, 8872–8881.
Mylonas, A.D., Brown, A.M., Carthew, T.L., McGrath, B., Purdie, D.M., Pandeya, N., Vecchio, P.C., Collins, L.G., Gardner, I.D., de Looze, F.J., Reymond, E.J., Suhrbier, A., 2002. Natural history of Ross River virus-induced epidemic polyarthritis. Med. J. Aust. 177, 356–360.
Peters, C., Dalrymple, J., 1990. Alphaviruses. In: Fields , B., Knipe, D., Chanock, R.M., et al., (Eds.), Virology, 2nd ed. Raven Press, New York, pp. 713–761.
Powers, A.M., Brault, A.C., Shirako, Y., Strauss, E.G., Kang, W., Strauss, J.H., Weaver, S.C., 2001. Evolutionary relationships and systematics of the alphaviruses. J. Virol. 75, 10118–10131.
Renner, C., Sacca, B., Moroder, L., 2004. Synthetic heterotrimeric collagen peptides as mimics of cell adhesion sites of the basement membrane. Biopolymers 76, 34–47.
Rousselle, P., Lunstrum, G.P., Keene, D.R., Burgeson, R.E., 1991. Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J. Cell Biol. 114, 567–576.
Rowley, M., Tait, B., Mackay, I.R., Cunningham, T., Phillips, B., 1986. Collagen antibodies in rheumatoid arthritis. Significance of antibodies to denatured collagen and their association with HLA-DR4. Arthritis Rheum. 29, 174–184.
Rubio, M.A., Sotillos, M., Jochems, G., Alvarez, V., Corbi, A.L., 1995. Monocyte activation: rapid induction of alpha 1/beta 1 (VLA-1) integrin expression by lipopolysaccharide and interferon-gamma. Eur. J. Immunol. 25, 2701–2705.
Smit, J.M., Waarts, B.L., Kimata, K., Klimstra, W.B., Bittman, R., Wilschut, J., 2002. Adaptation of alphaviruses to heparan sulfate: interaction of Sindbis and Semliki forest viruses with liposomes containing lipid-conjugated heparin. J. Virol. 76, 10128–10137.
Smith, T.J., Cheng, R.H., Olson, N.H., Peterson, P., Chase, E., Kuhn, R.J., Baker, T.S., 1995. Putative receptor binding sites on alphaviruses as visualized by cryoelectron microscopy. Proc. Natl. Acad. Sci. U.S.A. 92, 10648–10652.
Soden, M., Vasudevan, H., Roberts, B., Coelen, R., Hamlin, G., Vasudevan, S., La Brooy, J., 2000. Detection of viral ribonucleic acid and histologic analysis of inflamed synovium in Ross River virus infection. Arthritis Rheum. 43, 365–369.
Suhrbier, A., Linn, M.L., 2004. Clinical and pathologic aspects of arthritis due to Ross River virus and other alphaviruses. Curr. Opin. Rheumatol. 16, 374–379.
Sweet, M.J., Hume, D.A., 1995. RAW264 macrophages stably transfected with an HIV-1 LTR reporter gene provide a sensitive bioassay for analysis of signalling pathways in macrophages stimulated with lipopolysaccharide, TNF-alpha or taxol. J. Inflamm. 45, 126–135.
Tulla, M., Pentikainen, O.T., Viitasalo, T., Kapyla, J., Impola, U., Nykvist, P., Nissinen, L., Johnson, M.S., Heino, J., 2001. Selective binding of collagen subtypes by integrin alpha 1I, alpha 2I, and alpha 10I domains. J. Biol. Chem. 276, 48206–48212.
Ubol, S., Griffin, D.E., 1991. Identification of a putative alphavirus receptor on mouse neural cells. J. Virol. 65, 6913–6921.
Vandenberg, P., Kern, A., Ries, A., Luckenbill-Edds, L., Mann, K., Kuhn, K., 1991. Characterization of a type IV collagen major cell binding site with affinity to the alpha 1 beta 1 and the alpha 2 beta 1 integrins. J. Cell Biol. 113, 1475–1483.
Vrati, S., Faragher, S.G., Weir, R.C., Dalgarno, L., 1986. Ross River virus mutant with a deletion in the E2 gene: properties of the virion, virus-specific macromolecule synthesis, and attenuation of virulence for mice. Virology 151, 222–232.
Vrati, S., Fernon, C.A., Dalgarno, L., Weir, R.C., 1988. Location of a major antigenic site involved in Ross River virus neutralization. Virology 162, 346–353.
Wang, K.S., Schmaljohn, A.L., Kuhn, R.J., Strauss, J.H., 1991. Anti-idiotypic antibodies as probes for the Sindbis virus receptor. Virology 181, 694–702.
Wang, K.S., Kuhn, R.J., Strauss, E.G., Ou, S., Strauss, J.H., 1992. High-affinity laminin receptor is a receptor for Sindbis virus in mammalian cells. J. Virol. 66, 4992–5001.
Way, S.J., Lidbury, B.A., Banyer, J.L., 2002. Persistent Ross River virus infection of murine macrophages: an in vitro model for the study of viral relapse and immune modulation during long-term infection. Virology 301, 281–292.
White, M., 1993. Integrins as virus receptors. Curr. Biol. 3, 596–599.
Wu, T.T., Kabat, E.A., 1970. An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. J. Exp. Med. 132, 211–250.
Zhang, W., Heil, M., Kuhn, R.J., Baker, T.S., 2005. Heparin binding sites on Ross River virus revealed by electron cryo-microscopy. Virology 332, 511–518.
Ziegler-Heitbrock, H.W., Thiel, E., Futterer, A., Herzog, V., Wirtz, A., Riethmuller, G., 1988. Establishment of a human cell line (Mono Mac 6) with characteristics of mature monocytes. Int. J. Cancer 41, 456–461.